STUDIES ON NITROGEN FIXING BLUE-GREEN ALGAE IN A.R.E.

By

MOHAMED ALI EL-BOROLLOSY

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This Thesis has been Approved

By: Salah Taha

Sabry Abdel Ghaffar

S. A. Fahmy

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C O N T E N T S

INTRODUCTION

The blue-green algae, are perhaps the most striking nitrogen-fixing organisms yet discovered. They have involved an advanced self-supporting system, being capable of living photoautotrophically in an environment entirely free of combined nitrogen. Yet, there is little doubt that they are extremely primitive organisms. They comprise the only class of algae where nitrogen-fixation has been remarkably demonstrated, although claims for fixation by algae from other groups have not been without their advocates.

Blue-green algae are of world-wide distribution (Venkataraman, 1969), being particularly abundant in the moist tropics and semi-tropics where they may aggregate to form gelatinous masses over the soil surface, and contribute appreciably to the nitrogen status of the habitat.

Their importance in paddy fields, without any doubt, accounts for much of the research carried out on these organisms in the Far-East countries, particularly in India and Japan (Watanabe, 1951).

Little is known about the distribution and abundance of algal groups in Egyptian soils. Attempts have recently been started in Egypt to isolate some pure strains of blue-green algae from paddy fields at different localities, and whatever their ability to fix nitrogen, in comparison with some foreign efficient strains.

In this investigation local blue-green algae strains were isolated, identified and subjected to several trials for purification. The effect of some nutritional and environmental factors on N- fixation capacity, as well as the effect of inoculating the soil with the algae on the growth of rice plants were investigated.

REVIEW OF LITERATURE

Historical :

Algae are widely distributed in nature in sunny places on land, and also in water, both fresh and salty, from the arctic regions to the tropics. Pringsheim (1949) made an extensive historical review on algae. He mentioned that Famintzin (1871) was probably the first to emphasize the possibility of ascertaining the nutritive needs of an alga with the aid of solutions of inorganic salts, then a line of investigation pursued by Molish (1895, 1896) and Benecke (1898) with considerable success.

Miquel (1890 - 1892) developed devices for cultivating diatoms in an artificial media without securing pure cultures devoid of bacteria, which were first claimed by Richter (1903) and Chouat (1904).

The first steps to obtain bacteria free algae were taken by Beijerinck (1890, 1893), who adopted the technique devised by Koch for bacteria ten years earlier, and used gelatin for fixing germs at definite places. Klebs (1896), however, doubted whether Beijerinck's cultures were really free from bacteria. The introduction of agar-agar instead of gelatin by Tischutkin (1897) and Marshall Ward (1899) marked an important step foreward.

Chodat (1904, 9, 13) also used agar but employed Erlenmeyer flasks instead of the Petri dishes used by Tischutkin and Marshall Ward. This complicated the technique without contributing any advantage. His method has even been used as late as 1926 by Bristol-Roach in her otherwise very able experiments.

To understand the ecology of algae, an experiment was first carried out by Beijerinck (1901). He found that certain Cyanophyceae, such as species of Nostoc, Anabaena and Cylindrospermum, usually develop when soil are covered with a relatively large amounts of water, only enriched by a low concentration of phosphate. He called these Nostocaceae oligonitrophilic, and suggested that they can utilize atmospheric nitrogen. An indirect contribution of algae to the fixation of atmospheric nitrogen in soil, by supplying the free-living, N-fixing Azotobacter with carbohydrates, was suggested by soil microbiologists at the end of the last century (Kossowitsch, 1894). This idea was pursued afterwards by several investigators. Bouilhac

(1896) found that the association of Nostoc with N-fixing free-living bacteria made possible the simultaneous development of both, and increasing fixation of atmospheric nitrogen, in a N-free medium. Nakano (1917) found that more atmospheric nitrogen was fixed in flasks that contained mixed culture of algae and Azotobacter than in those containing Azotobacter alone. The work of Bouilhac (1896), Chodat (1904), and Pringsheim (1914) led progressively to the attainment of bacteria-free cultures of Cyanophyceae used further in physiological studies.

The pipette method for isolating single filaments or cells of algae was first recommended by Pringsheim (1921), then improved by Lwoff (1923, 1929).

Algal groups were found by Lund (1945) to flourish in fertile soils well supplied with basis, available phosphates and nitrates. Fenton (1943) noted that blue-green algae predominate over other algal groups in tropical soils.

In the last two decades, great interest was given for the blue-green algae, due to its ability to fix atmospheric nitrogen.

Classification :

Prescott (1969) stated that before 1800 algae were divided into four groups being: Conferva, Ulva, Chara and Fucus.

According to Tippo (1942) algae were classified to 7 phyla depending on morphology and pigmentation. The blue-green algae were suggested to belong to Phylum Cyanophyta.

The Cyanophyta contain several colourless forms. These bear a strong superficial resemblance to some bacteria. According to Smith (1950) Cohn as early as 1879 was the first to suggest that both bacteria (Schizomycetes) and blue-green algae (Schizophyceae) could be regarded as two classes of the Phylum Schizophyta.

According to Breed et al.(1957) the premitive organisms (bacteria, blue-green algae, Rickettsia and viruses) were separated from the Phylum Thallophyta and consequently a new Phylum Protophyta was suggested to include all these microorganisms and the blue-green algae are classified in the Class Schizophyceae.

The Schizophyceae are a remarkable group of organisms, which show some relationships with the other algae on one hand, and on the same time exhibit certain features in common with bacteria. Blue-green algae are unicellular, colonial, and filamentous organisms in which phycocyanin usually predominates. Pigments are located partly in the cell's peripheral region (chromoplasm), there being no chloroplast. Cells without an organized nucleus with nuclear membrane and nucleolus (Prescott, 1969).

Smith (1950) classified class Schizophyceae into five orders, which are briefly described in the following:

a) Without hormogonia :

I. Chroococcales: Unicellular or colonial (commonly palmelloid), multiplication by cell-devision and by endospores. Fam. Chroococcaceae, Cyanochloridaceae and Entophysalidaceae.

II. Chamaesiphonales: Unicellular or colonial, epiphytes or lithophytes, exhibiting marked polarity, multiplication by endospores or exospores.

Fam. Dermocarpaceae, Chamaesiphonaceae, Endonemataceae and Siphononemataceae.

III. Pleurocapsales: Heterotrichous filamentous types devoid of heterocysts, multiplication by endospores.

Fam. Pleurocapsaceae and Hyellaceae

b) <u>With hormogonia</u> :

IV. Nostocales: Fam. Oscillatoriaceae, Nostocaceae, Microchaetaceae, Rivulariaceae, Scytonemataceae and Brachytrichiceae.

V. Stigonematales: Fam. Pulvinulariaceae, Capsosiraceae, Stigonemataceae, Nostochopsidaceae and Loefgreniaceae.

Orders Nostocales and Stigonematales are the two largest groups of blue-green algae and both are characterised by the production of hormogonia. Nostocales are unbranched, or show false branching whereas true branching is characteristic of the Stigonematales.

The very important families including genera which fix atmospheric nitrogen are: Nostocaceae, Scytonemataceae, Stigonemataceae and Rivulariaceae.

Isolation and purification :

Unialgal cultures of filamentous algae can often be isolated by transferring a single filament to a suitable nutrient solution, by means of a special micropipettes (Pringsheim, 1921, and improved by Lwoff, 1929).

Pure cultures of filamentous algae have been obtained by shaking material in repeated changes of water, or by the agar plate method (Pringsheim, 1949).

As stated by Smith (1950) there is some confusion in the use of the term "pure culture". According to some authors, a pure culture is that which contains one species of alga, others understood it to be a culture of a one species of alga which is also free from other organisms including bacteria and fungi. To differentiate between the two, the term "unialgal culture" had been proposed to designate one which contains a single species of alga but which may contain other organisms. The term "pure culture" is reserved for one which contains a single species of alga and is absolutely free from other organisms.

Considerable difficulty is encountered in obtaining pure cultures of Schizophyceae. Separation of the